

# Preliminary Overview of the First Extensive Rust Resistance Screening Tests of *Pinus flexilis* and *Pinus aristata*

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Limber pine (*Pinus flexilis* James) and Rocky Mountain bristlecone pine (*P. aristata* Engelm.; hereafter referred to as bristlecone pine) are the dominant pines that occupy high elevation habitats of the southern Rockies. Bristlecone pine is primarily a subalpine and tree-line species while limber pine in the southern Rocky Mountains grows from 1600 m in the short grass steppe to over 3300 m elevation near the continental divide (see Schoettle 2004). These trees provide many ecosystem services including food for corvids, bears and squirrels, watershed protection, and picturesque gnarled tree forms on exposed sites. Both species are susceptible to infection by *Cronartium ribicola* J. C. Fisch., the non-native fungal pathogen that causes the lethal disease white pine blister rust (WPBR). WPBR has been present on limber pine since the 1970's in southern Wyoming and was first detected in northern Colorado in 1998 (Johnson and Jacobi 2000) and was discovered in southern Colorado infecting limber pine and bristlecone pine in 2003 (Blodgett and Sullivan 2004). The origin of the inoculum for the southern Colorado infection center is unclear, as it is over 200 km from the nearest known WPBR infections. Long distance transport of spores from

California is possible (Frank and others 2008) and may be responsible for initiating this southern Colorado infection center.

Early WPBR resistance testing by Hoff and others (1980) confirmed the occurrence of resistance in both limber and bristlecone pines. Kinloch and Dupper (2002) reported the occurrence of an apparent hypersensitive (HR) needle-based reaction to WPBR in limber pine, similar in gross phenotype to a resistance controlled by a single dominant gene in western white pine (*P. monticola* Douglas ex D. Don) and sugar pine (*P. lambertiana* Douglas). However, Kinloch and Dupper (2002) were unable to confirm inheritance of the complete resistance trait in limber pine as their sample was a bulk seed collection from several trees.

After making seed collections, we initiated the first extensive studies of resistance to WPBR in limber pine and bristlecone pine in family structures (table 1). Our studies are quantifying the frequency of resistances within and among families and populations throughout the southern Rockies. In collaboration with Dorena Genetic Resource Center (DGRC; Cottage Grove, OR) and Institute of Forest

**Table 1.** Rust resistance studies ongoing for southern Rocky Mountain sources of limber pine and bristlecone pine.

Species	Exploring Resistance Type	Families in Testing (number)	Rust Inoculum Sources	Sow Year	Inoculation Year
<i>P. flexilis</i>	Complete	113 <sup>a</sup>	1	2006, 2007	2006, 2007
		153 <sup>a</sup>	1	2010	2010
		31 (bulk lots) <sup>a</sup>	1	2009, 2010	2009, 2010
	Partial	74 <sup>a</sup>	2 <sup>b</sup>	2007	2008
<i>P. aristata</i>	Complete	4 <sup>a</sup>	1	2009	2009
	Partial	189 <sup>a</sup>	1	2002	2005, 2009
		109 <sup>c</sup>	1	2002	2004

<sup>a</sup> Studies lead by Rocky Mountain Research Station (RMRS, Fort Collins, CO) in collaboration with Dorena Genetic Resources Center (DGRC; Cottage Grove, OR).

<sup>b</sup> Inoculum from wild-type eastern Oregon sources was used in each study at DGRC. In addition, for the partial resistant test of limber pine, two trials were inoculated with different geographic sources of rust: a full set of replicates were inoculated with wild-type *C. ribicola* from eastern Oregon and a second set of replicates were inoculated with *C. ribicola* from western Oregon that contained the Champion Mine (vcr2) strain that is virulent to the HR-type simply inherited complete resistance in western white pine.

<sup>c</sup> Study initiated by RMRS (Fort Collins, CO) and lead by Pacific Southwest Station Institute of Forest Genetics (Placerville, CA). The families in this study are a subset of those included in the 189-family test at DGRC.



**Figure 1.** Post-inoculation disease phenology on limber pine seedlings inoculated at 5-months old at Dorena Genetic Resource center. **A.** Needle lesions (spots) visible at 3 months; **B.** incipient cankers beginning to show by 4 to 6 months; **C.** *spermatia* (pynchia) evident at 10 months; **D.** aecia present at 18 months.

Genetics (Placerville, CA), we are conducting short- and long-duration tests to explore complete and partial resistance mechanisms (Table 1). Results presented here examine some preliminary findings for those trials conducted at DGRC.

Consistent seedling culture and effective seedling inoculation of both species with *C. ribicola* has been achieved. At DGRC, inoculation densities of 3,500 to 9,500 basidiospores/cm<sup>2</sup> produce very high infection frequencies (>99% of seedlings with needle infections) for 3-, 5- and 17-month-old seedlings of limber pine. Five-month-old bristlecone pine responded similarly. In 2005, for the large trial at DGRC of older, 36-month old bristlecone pine seedlings an inoculum density of approximately 14,000 basidiospores/cm<sup>2</sup> was used. Quantifying infection frequencies for bristlecone pine is more complex as needle lesions are less obvious on this species (see below).

A diversity of needle lesions (infection spots) develop on limber pine; they range in color from golden to deep red with some lesions expanding over time while others remaining more discrete. In the greenhouse environment, needle lesions become easily visible in as little as 3 months after inoculation for the young material and reliably by 4-8 months after inoculation for the older material. In the outdoor environment, the appearance of needle lesions was generally slightly later, similar to western white pine and whitebark pine (*P. albicaulis* Engelm.) at DGRC. The phenology of cankering on limber pine seedlings was also typical (fig. 1). In the greenhouse environment, stem symptoms (lesions) became visible on the younger limber pine seedlings within 4 months following inoculations and continued to appear and develop over the next 18 months. *C. ribicola* spermatia and aecia developed on both younger and older inoculated limber pine seedlings.



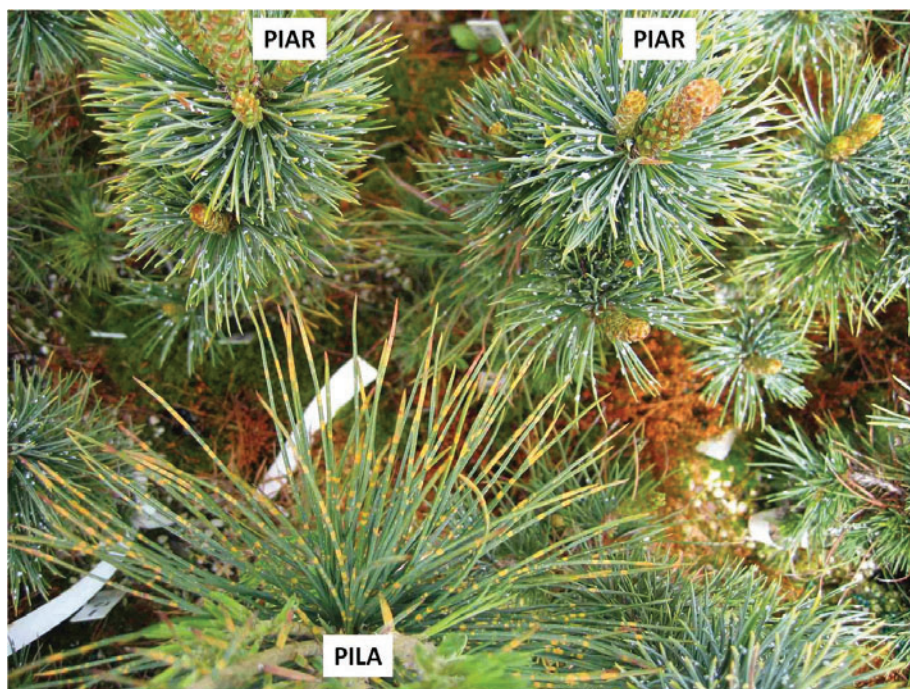
Complete resistance to WPBR in limber pine has been evaluated in progeny of 113 limber pine seedtrees (families) from 13 populations across the Southern Rockies. The frequency of resistance varied among populations from 1 to 29 percent and among families from 0 to 100 percent (Schoettle, Snieszko, and Burns, unpublished data). Assessment of the frequency of the partial resistance mechanisms in limber pine is underway at DGRC (Schoettle, Snieszko, Pineda-Bovin and Burns, in progress). The partial resistance testing utilizes two inoculum sources: a full set of replicates were inoculated with wild-type *C. ribicola* from eastern Oregon and a second set of replicates were inoculated with *C. ribicola* from western Oregon that contained the Champion Mine (vcr2) strain that is virulent to the HR-type simply inherited complete resistance in western white pine. Early results suggest that needle lesions and cankering develop earlier when the trees are exposed to the vcr2 strain of *C. ribicola* yet over time the families appear to be responding similarly to both inoculums (Schoettle, Snieszko, Pineda-Bovin and Burns, unpublished data). Two years after inoculations, many susceptible seedlings have died yet some evidence of tolerance to cankering has been observed suggesting the presence of at least one partial resistance mechanisms in limber pine.

An inoculation trial of young bristlecone pine seedlings (5-months old) revealed an abundance of needle lesions and a similar phenology of disease symptom development as limber pine and other species. However, needle lesions on the older 36-month-old bristlecone pines in the 2005 inoculation trial were less obvious. Needle lesions on the primary needles of bristlecone (usually near the lower portions of the stem) were easily visible nine months after inoculation but needle lesions on secondary needles were less clear than on the control seedlings (fig. 2). The control seedlings of susceptible sugar pine and western white pine, which were

dispersed throughout the bristlecone trial, displayed numerous needle lesions and the expected phenology of disease expression verifying that the inoculation was successful in challenging all the seedlings with the pathogen. Stem symptoms, spermatia and aecia, developed on the susceptible older bristlecone pine seedlings even though macroscopic needle lesions were not always apparent as has been observed on other species. These seedlings were grown outdoors for two years prior to inoculation so the secondary needles were quite tough, which may have contributed to masking hyphal growth under the epidermis.

Stem lesions (cankers) developed later in bristlecone pine than the control seedlings of western white pine and sugar pine in the screening test in the DGRC test. This is consistent with field observations of latent periods as long as 8 to 14 years (see fig 3) or longer between needle infection and aecia production on some bristlecone pine trees compared to the more typical 1 to 3 year period for other species.

Preliminary assessments suggest that three years after rust inoculation the frequency of disease-free progeny from 189 bristlecone pine seedtrees from 11 populations in Colorado varies among populations from 17 to 60% and among families from 0 to 92 percent (Schoettle, Snieszko, Kegley, and Burns, in progress) with an overall frequency of 37 percent. Assessments of these seedlings continue at DGRC and frequencies of partial resistances are being estimated. The screening trial at the Institute of Forest Genetics (Placerville, CA) used younger seedlings grown in a greenhouse and early results (one year post-inoculation with rust) suggest 22 percent of the inoculated seedlings showed no symptoms of disease (Vogler and others 2006). Even with the difference in seedling culture and inoculums source, preliminary results between the two studies appear consistent and correlated. Further comparison of family performance differences is ongoing.



**Figure 2.** Comparison of bristlecone (PIAR) with a sugar pine (PILA; *Pinus lambertiana*) control seedling nine months post-inoculation; both seedlings were inoculated at the same time under the same conditions at Dorena Genetic Resource Center. Note the abundant visible needle lesions on the sugar pine seedling and the absence of obvious needle lesions on the bristlecone pine. The white dots on the bristlecone needles are resin and typical for this species; they are not a symptom of stress or *C. ribicola* infection.



**Figure 3.** *Pinus aristata* branch from a mature tree on Mosca Pass collected in 2004. The pins are placed at the bud scale scars which denote the end/beginning of each annual branch growth segment. The year listed is the year that the branch segment (and any attached needles for the foliated portion) was formed. This is not a particularly vigorous branch and its annual extension growth has decreased since 1990. Current year needles plus six previous years of needles are retained on this branch. The first year that *C. ribicola* aecia formed on the growth segment formed in 1990 was 2004, the year the shoot was collected. If we assume that needle retention is consistent from year to year (and the infection occurred through the needles as it typically does), and we assume the branch was infected in 1996, at which time the needles formed in 1990 were 6 years old, the latent period between infection and aecia formation would be 8 years. Alternatively, if the year of needle infection was earlier than 1996, when the needles formed in 1990 were younger, the latent period could be up to 14 years for this branch. Therefore, the latent period for this shoot is 8 to 14 years. However, without understanding the susceptibility of different needle age classes to infection or the length of the latent period, we are unable to determine which year between 1990 and 1996 rust infected the branch.

In summary, preliminary results confirm the occurrence of family-based resistances in both limber pine and bristlecone pine from the southern Rockies. Further examination of the infection at the needle level, using histological techniques, is ongoing in collaboration with Oregon State University to determine if the reaction in limber pine is similar to the HR-type in western white pine. No evidence of HR-type complete resistance was observed in the bristlecone pine trial at the Institute of Forest Genetics (Vogler and others 2006) or in the small 2009 greenhouse trial at DGRC. Preliminary results from the partial resistance tests suggest multiple resistance mechanisms are present in limber pine and bristlecone pine. These data also suggest geographic variation in the distribution of resistances and on-going studies are exploring these relationships further in both species.

Results from this research are being integrated with ecological and gene conservation efforts to develop proactive interventions to sustain limber pine and Rocky Mountain bristlecone pine populations into the future (Schoettle and Sniezko 2007; Schoettle and others, The Proactive Strategy for Sustaining Five-Needle Pine Populations, this proceedings; Keane and Schoettle this proceedings). The rust resistance studies provide baseline information on the resistance mechanisms and frequencies of WPBR resistances for populations of limber pine and bristlecone pine in the Southern Rockies before they are invaded or severely impacted by WPBR. Several detailed syntheses of results for the different trials are underway and other trials are ongoing. These first studies serve to refine the screening methodologies for limber and Rocky Mountain bristlecone pines and provide the first family-based estimates of the frequencies of resistances for these species.

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## References

- Blodgett, J. T.; Sullivan, K. F. 2004. First report of white pine blister rust on Rocky Mountain Bristlecone pine. *Plant Dis.* 88: 311.
- Frank, K. L.; Geils, B. W.; Kalkstein, L. S.; Thistle Jr., H. W. 2008. Synoptic climatology of the long-distance dispersal of white pine blister rust II. Combination of surface and upper-level conditions. *Int. J. Biometeorol.* 52: 653–666.
- Hoff, R. J.; Bingham, R. T.; McDonald, G. I. 1980. Relative blister rust resistance of white pines. *European Journal of Forest Pathology.* 10: 307–316.
- Johnson, D. W.; Jacobi, W. R. 2000. First report of white pine blister rust in Colorado. *Plant Disease.* 84: 595.
- Kinloch, B. B., Jr.; Dupper, G. E. 2002. Genetic specificity in the white pine/blister rust pathosystem. *Phytopathology.* 92: 278–280.
- Schoettle, A. W. 2004. Ecological roles of five-needle pines in Colorado: Potential consequences of their loss. In: Sniezko, R.; Samman, S.; Schlarbaum, S.; Kriebel, H. Eds. *Breeding and genetic resources of five-needle pines: growth adaptability and pest resistance.* 2001 July 24–25; Medford, OR. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. Pp. 124–135.
- Schoettle, A. W.; Sniezko, R. A. 2007. Proactive intervention to sustain high elevation pine ecosystems threatened by white pine blister rust. *Journal of Forest Research.* 12(5): 327–336. <http://www.springerlink.com/content/9v91t44278w74430/fulltext.pdf>
- Vogler, D. R.; Delfino-Mix, A.; Schoettle, A. W. 2006. White pine blister rust in high-elevation white pines: screening for simply-inherited, hypersensitive resistance. In: Guyon, J. C., compiler. *Proceedings of the 53th Western International Forest Disease Work Conference; 2005 September 26–30; Jackson, WY.* Ogden UT: USDA Forest Service, Intermountain Region. Pp 73–82.

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